Supporting Information

Surface Enhanced Raman Scattering Tags for Rapid and Homogeneous Detection of Circulating Tumor Cells in Presence of Human Whole Blood

Michael Y. Sha*, Hongxia Xu, Michael J. Natan and Remy Cromer
Oxonica Inc. 325 E.. Middlefield Rd, Mountain View, CA 94043.
* mike_sha168@yahoo.com

MATERIALS AND METHODS

Cell line and cell culture

SKBR3 (HTB-30) cell line was purchased from ATCC. The vial was thawed with gentle agitation in a 37°C water bath. The vial contents were transferred to a centrifuge tube containing 9.0 ml complete culture medium and spun at approximately 125 x g for 5 to 7 minutes. The cell pellet was re-suspended with 9 ml of McCoy5 with10% FBS and dispensed into a 25 cm^2 or a 75 cm^2 culture flask. Prior to the addition of the vial contents, the culture vessel containing the complete growth medium has been placed into the incubator for at least 15 minutes to allow the medium maintaining its normal pH (7.0 to 7.6). The cell culture was incubated in an incubator at 37°C with 5% CO2 in air atmosphere.

Magnetic bead conjugated with EpCAM antibodies

4.5 um Dynalbead (Collection Epithelial Enrich, Cat number 162-03) was purchased from Invitrogen Inc. This magnetic bead is already conjugated with EpCAM antibody.

Nanoplex ™ biotag synthesis and functionalization

The synthesis of the Nanoplex™ Biotags has been described by Mulvaney et al [1]. Briefly, a 50 nm spherical Au colloid is coated first with a layer containing both a glass precursor silane and the Raman label molecule. A thin (a few nanometers) silica coating

is grown then by slow polymerization of Na silicate providing encapsulation of the Raman reporter molecule on the gold surface. The thin glass shell is subsequently thickened up to 20-30 nm by using a mixture of Ethanol, water, tetraethylorthosilicate (TEOS) and Ammonia. To facilitate the use of these nanoparticles as a biological tag, the glass surface is further functionalized with bioreactive groups such as thiols or amines. The Nanoplex^{TM} Biotags used in this work were thiolated by replacing 5% of the TEOS with mercaptopropyltrimethoxysilane (MPTMS).

Conjugation of her2 antibody or Streptavidin to Nanoplex ^{TM} biotags

1.5 ml of thiol-modified Nanoplex biotag (OD 24) was mixed with 250 µl of 50 mM phosphate buffer (pH 7.15) in a 2 ml Eppendorf tube. 120 µl of mouse anti-HER2 (c-erbB-2) antibody (5 mg/ml, Clone: TAB250, epitope: extracelluar domain, Cat number 28-003Z, Invitrogen inc. CA) and 120 µl of freshly prepared sulfo-SMCC (5 mg/ml in H_2O) were then added to the tube, reacted with gentle mixing for 2.5 hours at room temperature. After incubation, 100 µl of a solution consisting of 10% BSA and 30 mg/ml MESA (in conjugation buffer) was added to the reaction tube and incubated at room temperature for 45 min for quenching the excess maleimide groups. For purifying the conjugates, the reaction mixture was centrifuged at 1000 RCF for 15 min. After removing the supernatant, the pellet was resuspended in 1.5 ml of resuspension buffer (20 mM phosphate, pH 7.5/0.1% BSA/0.05% sodium azide). The centrifugation/resuspension steps were repeated for four times and the particles were then resuspended to desired concentration. Same procedure was used for Streptavidin conjugated SERS tags.

Labeling SK-BR3 cells with Nanoplex ^{TM} biotag for Immunostaining

The procedure had been described before. [2] SKBR3 cells were cultured in McCoy5A medium with 10% fetal bovine serum on chamber slide. The cells at 60-70% confluence

were washed with PBS three times, 1 min each. Then, the cells were fixed with 3.7% formaldehyde/PBS for 10 min at room temperature and washed with PBS for three times. After removing of wash buffer, the cells were blocked with 1% BSA/PBS for 20 min at room temperature, incubated with 1 μg/ml mouse anti-her2 antibodies (diluted with 1% BSA/PBS) for 30 min at room temperature and washed with PBS for 3 times. After incubating with 1 μg/ml biotinylated anti-mouse IgG for 30 min, the cells were washed with PBS three times and incubated with 50 μl 2X Streptavidin conjugated SERS tags at room temperature for 1 hr. Followed by washing with PBS for three times, the cells were incubated with 1:500 diluted Hoechest for 5 min, washed with PBS for three times and mounted with coverslip using 90% glycerol in PBS and sealed up the edge of the coverslip with nail polish. The slide was examined under a fluorescence microscope.

Nanoplex ™ biotag direct cell assay for detection of circulating tumor cell

The SKBR3 cells were grown to 70%–80% confluence in McCoy5A with 10% FBS and harvested in PBS buffer with two time wash. The cells were counted by hemocytometer and diluted accordingly. Experiments were performed either in a buffer system or in a whole blood system. For study in the buffer system, we added 100 μl SKBR3 cells in PBS to the mixture of 50 μl Neptune Assay Diluent (AD3 buffer, Immunochemistry Technologies, LLC. MN 55431), 1 μl magnetic bead-EpCAM ($5X10^5$ beads) and 4 μl 20x Nanoplex ™ biotag conjugated with her2 antibodies and incubated for 30 min at room temperature. The incubation mixture was pulled down under the magnetic power and the Raman signal was acquired with QE65000 Raman spectrum instrument. For the whole blood system, we added 50 μl SKBR3 cells into the mixture of 100 μl whole blood sample, 50 μl AD3 buffer, 1 μl magnetic bead-EpCAM ($5X10^5$ beads) and 4 μl 20x Nanoplex ™ biotag conjugated with her2 antibodies together and

incubated for 30 min at room temperature. Each data point was collected from triple sample. Each test was repeated three times.

Raman instrument Nanoplex™ Reader design

A Raman spectrometer instrument compatible with the assay format, consisting of an optical system and sample tube holder was developed in house. The sample holder includes a magnetic assembly that produces a focused magnetic field at a precise location on the wall of the sample tube. This location is coincident with the focal point of the Raman optical sensor. In this way, magnetic particles are pre-concentrated at this location on the tube such that quantitative measurement of Nanoplex™ biotags associated with the magnetic beads is facilitated. The optical measurement system comprises a Raman spectrometer with an excitation wavelength of 785 nm (Ahura Corp. ECL 785-325 laser module) configured to deliver approximately 70 mW power.1000 ms acquisition time, 300 μm beam diameter for sample detection. An Inphotonics filter head and Ocean optics QE65000 spectrometer complete the optical train.

Data analysis

SensenSee ™ software was developed in house. The SERS signal was calculated from a Nanoplex biotag (S-420) which one of peak in 1600 Raman Shift (cm -1). The limits of detection (LOD) was calculated base on “background signal + 3x standard deviation”.

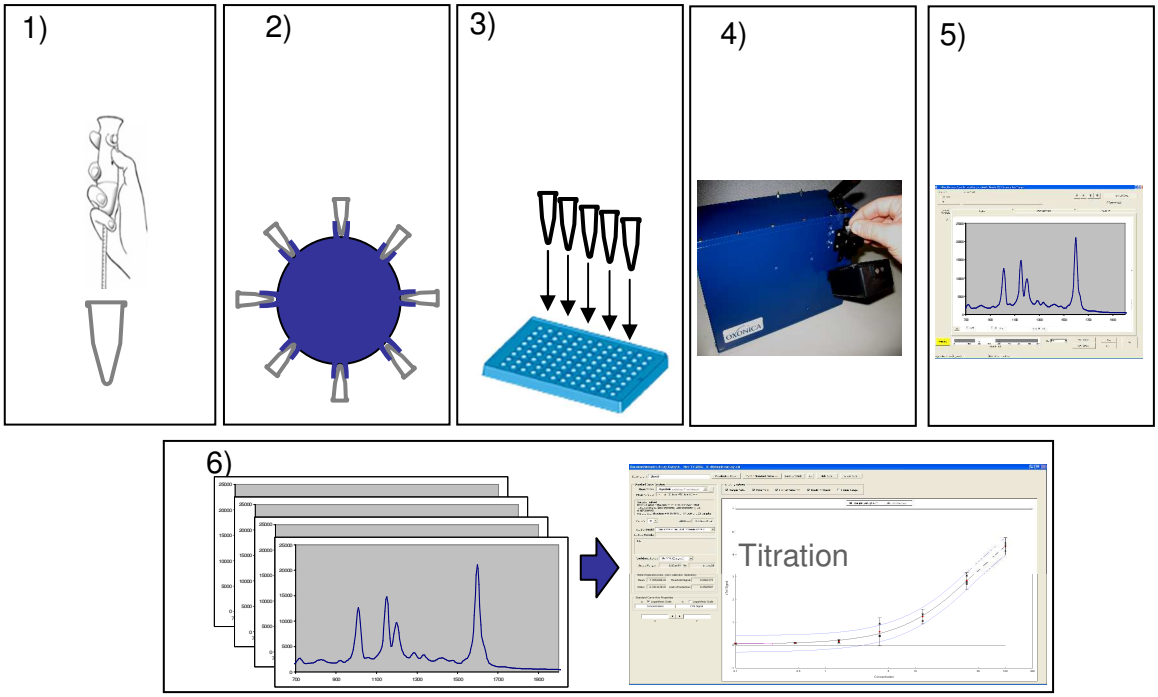

Figure S1. CTC Detection Assay Process. 1). Sample mixed with SERS tag and magnetic beads; 2) Incubation; 3). Place in 96 well magnetic plate; 4) Insert the sample tube in the Nanoplex Reader; 5) Capture spectra on computer; 6) Data process.

Reference List

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3. Sun, L.; Sung, K. B.; Dentinger, C.; Lutz, B.; Nguyen, L.; Zhang, J. W.; Qin, H. Y.; Yamakawa, M.; Cao, M. Q.; Lu, Y.; Chmura, A. J.; Zhu, J.; Su, X.; Berlin, A. A.; Chan, S.; Knudsen, B. *Nano Lett.* **2007**, *7*, 351-56.

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